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Final Report

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Experimental progress has been reported in detail in a number of semi-annual reports, the last of which was prepared on March 14, 1962. The major findings have been published in the papers listed below:

1. Hess, M.E., Shanfeld, J. and Haugaard, N. The influence of Sympathetic Activity on Rat Heart Phosphorylase. J. Pharmacol. 131, 143, 1961.
2. Hess, M.E., Shanfeld, J. and Haugaard, N. The Role of the Autonomic Nervous System in the Regulation of Heart Phosphorylase in the Open-Chest Rat. J. Pharmacol. 135, 191, 1962.
3. Haugaard, N., Inesi, G. and Haugaard, E.S. Glucose Oxidation and High Energy Phosphate Production in Rat Heart Homogenates. Circulation Research 11, 381, 1962.
4. Inesi, G., Pekkarinen, A., Hess, M.E., Shanfeld, J. and Haugaard, N. The Influence of Bretylium on the Action of Reserpine and Mc-Neil-A-343. Biochem. Pharmacol. 11, 1089, 1962.

   a. Glucose Oxidation and ATP Formation in Cardiac Tissue.

      Experimental conditions have been established for the study of glucose oxidation and phosphorylation of AMP in cell-free rat heart homogenates. In the presence of glucose oxidation the system is capable of synthesizing ADP and ATP from AMP at a high rate. In the absence of added AMP rapid glucose oxidation also occurs despite the presence of extremely low levels of ATP. It was concluded that the high concentration of ATP present in heart tissue in vivo

is not necessary for efficient glucose oxidation (3).

b. Action of Drugs on Heart Phosphorylase Activity

In previous communications from this laboratory it has been reported that the increased force of contraction following administration of catecholamines to the perfused rat heart is associated with an increase in the activity of the enzyme phosphorylase a.

In the present investigation it was demonstrated that in the intact rat cardiac phosphorylase activity was influenced by conditions or drugs which affected the activity of the sympathetic nervous system.

The activity of phosphorylase a in the heart was found to be extremely high in decapitated rats. The high values of phosphorylase a following decapitation were found to be significantly depressed in animals pre-treated with hexamethonium, bretylium or reserpine, drugs which in different ways inhibit sympathetic activity. Anesthesia with ether or pentobarbital was also found to depress significantly the stimulation of phosphorylase produced by decapitation. It was noted that the level of phosphorylase a activity was much higher in hearts from intact animals than in hearts perfused with Locke solution. This indicates that in vivo the autonomic nervous system or blood constituents influence the activity of this enzyme. It was concluded from this investigation that in the intact animal cardiac phosphorylase a is influenced by the sympathetic nervous system (1).

The open-chest rat preparation was used in further studies of heart function and phosphorylase activity. It was found that the increase in force of contraction

produced by the ganglion-stimulating drug DMPP was associated with an increase in activity of cardiac phosphorylase a. On the other hand, there was a significant decrease in phosphorylase a following administration of acetylcholine or vagal stimulation. It was concluded that cardiac phosphorylase activity is under the influence of both divivisions of the autonomic nervous system (2).

c. The Effect of Drugs on Heart Catecholamine Content

In studies of the action of drugs on heart catecholamine content it was demonstrated that bretylium markedly inhibited the depletion of norepinephrine in the heart produced by reserpine. Bretylium itself had little effect on heart catecholamines. It was also found that bretylium effectively blocked the increase in blood pressure and cardiac phosphorylase activity produced by administration of the ganglion-stimulating drug McNeil-A-343(4). These findings are in agreement with the view that bretylium produces its effects by preventing the release of norepinephrine from nerve endings.

CURRENT STUDIES

The investigations described above being continued and extended.

a) Glucose Oxidation and Phosphorylation.

The "glucose oxidation system" has been used for studies of the action of methylxanthines on metabolism. These compounds have been found to inhibit the net synthesis of ATP by rat heart homogenates. Their action is similar to that of low concentrations of calcium and their effectiveness is significantly reduced by ethylenediamine tetra-acetic acid (EDTA). These observations indicate

that some of the metabolic effects of the methylxanthines may be mediated by an action of these drugs on tissue calcium. Glucose oxidation and ATP formation is being studied in brain homogenates in preparation for an investigation of the metabolic effect of drugs in this tissue.

b) Phosphorylase

A study of the action of theophylline on function and metabolism has been completed. In the rat diaphragm in vitro theophylline itself had no effect on the activity of phosphorylase a. However, it potentiated the action of epinephrine on this enzyme. In the perfused heart the increase in force of contraction, produced by theophylline, was associated with an increase in phosphorylase a. At the dose levels studied, theophylline potentiated the biochemical but not the functional effects of epinephrine.

Studies on the action of drugs on heart function and phosphorylase activity are being continued with the perfused heart and the open-chest rat preparation. An investigation is also being made of the action of sympathetic, parasympathetic and adrenergic blocking agents on phosphorylase activity of tissue preparations incubated in vitro. It is of interest that the sympathomimetic amine, methoxamine, is capable of causing a complete block of the action of epinephrine on heart and diaphragm phosphorylase. These observations agree with the recent view that methoxamine may be considered to be an adrenergic β-blocking agent.

c) Electron Microscopic Localization of Cholinesterases

Of the various histochemical procedures which have been developed for the histochemical localization of cholinesterases (ChE's) by light microscopy, the thiolacetic acid (ThAc) appears to offer

the greatest promise for application to electron microscopy. Its major advantage is the potentiality of having all reagents involved in the immediate localizing reaction in uncharged form in sufficient concentrations to penetrate cell membranes, lipid myelin sheaths, and other barriers at sufficient rates to minimise diffusion artifacts; this consideration is unimportant in light microscopy, where sections are cut prior to exposure to the incubation medium. Its major limitation is lack of specificity, but this should be controllable by selective inhibitors. Several modifications of this method have been screened by light microscopy and tested with the electron microscope in this laboratory by Dr. Richard Davis and Dr. Koelle over the past two years. Considerable improvement over published results has been obtained, but this is not yet sufficient to warrant publication.